FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)
101215-63	09/856099

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP99/09747	22 November 1999 (22.11.99)	20 November 1998 (20.11.98)

TITLE OF INVENTION

Device and Method for the Parallel Separation of Substances by Liquid Chromatography

APPLICANT(S) FOR DO/EO/US

Lutz Muller-Kuhrt; Ralf God; Holger Gumm; and Jorg Binkele

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☐ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ A copy of the International Search Report (PCT/ISA/210).
8. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☐ Other items or information:

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/856099	PCT/EP99/09747	101215-63

21. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :		CALCULATIONS PTO USE ONLY
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO	$1,000.00	
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO	$860.00	
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$710.00	
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$690.00	
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)	$100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =		$860.00
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		$130.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	- 20 =	0	x $18.00	$0.00	
Independent claims	- 3 =	0	x $80.00	$0.00	
Multiple Dependent Claims **(check if applicable)**.			☐	$0.00	
TOTAL OF ABOVE CALCULATIONS			=	$990.00	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)**.			☒	$495.00	
SUBTOTAL			=	$495.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).			+	$130.00	
TOTAL NATIONAL FEE			=	$625.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☐	$0.00	
TOTAL FEES ENCLOSED			=	$625.00	
				Amount to be: refunded	$
				charged	$

☐ A check in the amount of to cover the above fees is enclosed.

☒ Please charge my Deposit Account No. **14-1263** in the amount of **$625.00** to cover the above fees.
A duplicate copy of this sheet is enclosed.

☐ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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27387
PATENT TRADEMARK OFFICE

SIGNATURE

Bruce S. Londa
NAME

33-531
REGISTRATION NUMBER

May 17, 2001
DATE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Atty's Docket No. 101215-64

EXAMINER :

GROUP ART UNIT :

APPLICANT : Lutz Müller-Kuhrt et al.

APPLN. NUMBER : 09/856,099

FILED : May 17, 2001

FOR : Apparatus and Method for the Parallel, Liquid Chromatographic Separation of Substances

SECOND PRELIMINARY AMENDMENT

Hon. Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Prior to examination, please amend the application as follows:

IN THE SPECIFICATION

Page 1, after line 2 (as amended under Article 34), please insert --Background of the Invention--;

Page 5, before line 6 (as amended under Article 34), please insert --Summary of the Invention--;

Page 5, before line 12 (as originally filed), please insert --Brief Description of the Drawings--; and

Page 7, before line 1 (as amended under Article 34), please insert --Description of the Preferred Embodiment--.

IN THE CLAIMS

We take this opportunity to provide a marked-up version and clean copy of the claims as amended in the preliminary amendment filed with the application on May 17, 2001.

REMARKS

The above amendments were made to place the application into proper United States Patent Format.

Respectfully Submitted,

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Amended Claims

1. An apparatus for the liquid chromatographic separation of substances under pressure, for which at least several liquid chromatographic separating lines (17), which are disposed in parallel, are supplied by a single pumping unit in the form of one or two pumps (3, 4) and, in the region, when the samples are supplied, are combined with a sample holding system (5) and an injection system (18) as well as, in the detection region, with a detector (13), connected with an evaluation and control unit (16), wherein the liquid chromatographic separation lines (17) have a separate flow control unit (10, 12, 12.1, 19), the flow regulating units (10, 12, 19) consisting of a flow controller (10.1 - 10.8), a total pressure meter (19) and flow meters (12.1 - 12.8).

2. The apparatus of claim 1, wherein the flow regulating units (10, 12, 19) in each separating line (17) can be controlled by software and/or hardware.

3. (amended) The apparatus of ~~claims 1 to 2~~ claim 1, wherein flow regulators (10) and flow meters (12) are disposed at different places in a separating line (17).

4. (amende) The apparatus of ~~claims 1 or 2~~ claim 1, wherein flow regulators (10) and flow meters (12) are disposed compactly in one place in the separating line (17).

5. (amended) The apparatus of ~~claims 1 to 4~~ claim 1, wherein the flow regulator unit (10, 12, 19) is disposed

in front of or behind the separating columns (11.1 to 11.8).

6. (amended) The apparatus of ~~claims 1 to 5~~ claim 1, wherein the total pressure meter (19) is disposed on the output side of the pump (3, 4).

7. (amended) The apparatus of ~~one of the claims 1 to 6~~ claim 1, wherein the sample holding system (5) is connected with at least several parallel sample holding lines over at least several injection ports (6) and injection valves (9) and sample loops (7) of the multi-parallel injection system (18) are connected with at least several separating columns (11.1 to 11.8), which are coupled with a detector (13), which has several determination channels.

8. (amended) The apparatus of ~~one of the claims 1 to 7~~ claim 1, wherein the separating columns (11.1 to 11.8) are combined compactly into a battery of separating columns (11).

9. (amended) The apparatus of ~~one of the claims 1 to 8~~ claim 1, wherein each injection valve (9) is disposed before the separating columns (11.1 to 11.8).

10. (amended) The apparatus of ~~one of the claims 1 to 9~~ claim 1, wherein each injection valve (9) is constructed as a multiple way valve.

11. (amended) The apparatus of ~~one of the claims 1 to 10~~ claim 1, wherein each injection valve (9.1 to 9.8)

has switching possibilities to an injection port (6), to a sample loop (7), to the pumps (3, 4), to a waste collector (8) and to a separating column (11.1 to 11.8).

12. (amended) The apparatus of ~~one of the claims 1 to 11~~ claim 1, wherein the separating lines (17.1 to 17.10) have a separating column and a solid phase extraction unit (13), which are coupled with further pumps (21, 22).

13. The apparatus of claim 12, wherein a multiple way valve, which can be connected with the solid phase extraction unit (23), the multi-parallel fraction output unit (24) and the waste collector (14), is disposed in the end region of the solid phase extraction unit (23).

14. (amended) The apparatus of ~~one of the claims 12 or 13~~ claim 12, wherein the solid phase extraction units (23) have at least two fractionating columns each.

15. (amended) The apparatus of ~~one of the claims 12 to 14~~ claim 12, wherein the solid phase extraction units (23) have between 10 and 50 fractionating columns.

16. A method for the liquid chromatograp[hic separation of substances under pressure, for which several samples, which are to be separated, are supplied simultaneously to at least several separating columns (11) and, subsequently, a detection and selection takes place simultaneously and in parallel, wherein the separating lines (17) are calibrated with respect to the retention times by means of a calibration sample and, after the

individual retention times have been determined, are adjusted to the same retention time by control with flow regulators (10) on the basis of data from flow meters (12) and initial pressure meters (19).

17. The method of claim 16, wherein the ratio of the total pressure to the volume flow to the respective separating line is used as actual value for indirectly controlling the volume flow.

Amended Claims - Clean Copy

1. An apparatus for the liquid chromatographic separation of substances under pressure, for which at least several liquid chromatographic separating lines (17), which are disposed in parallel, are supplied by a single pumping unit in the form of one or two pumps (3, 4) and, in the region, when the samples are supplied, are combined with a sample holding system (5) and an injection system (18) as well as, in the detection region, with a detector (13), connected with an evaluation and control unit (16), wherein the liquid chromatographic separation lines (17) have a separate flow control unit (10, 12, 12.1, 19), the flow regulating units (10, 12, 19) consisting of a flow controller (10.1 - 10.8), a total pressure meter (19) and flow meters (12.1 - 12.8).

2. The apparatus of claim 1, wherein the flow regulating units (10, 12, 19) in each separating line (17) can be controlled by software and/or hardware.

3. (amended) The apparatus of claim 1, wherein flow regulators (10) and flow meters (12) are disposed at different places in a separating line (17).

4. (amende) The apparatus of claim 1, wherein flow regulators (10) and flow meters (12) are disposed compactly in one place in the separating line (17).

5. (amended) The apparatus of claim 1, wherein the flow regulator unit (10, 12, 19) is disposed in front of or behind the separating columns (11.1 to 11.8).

6. (amended) The apparatus of claim 1, wherein the total pressure meter (19) is disposed on the output side of the pump (3, 4).

7. (amended) The apparatus of claim 1, wherein the sample holding system (5) is connected with at least several parallel sample holding lines over at least several injection ports (6) and injection valves (9) and sample loops (7) of the multi-parallel injection system (18) are connected with at least several separating columns (11.1 to 11.8), which are coupled with a detector (13), which has several determination channels.

8. (amended) The apparatus of claim 1, wherein the separating columns (11.1 to 11.8) are combined compactly into a battery of separating columns (11).

9.(amended) The apparatus of claim 1, wherein each injection valve (9) is disposed before the separating columns (11.1 to 11.8).

10. (amended) The apparatus of claim 1, wherein each injection valve (9) is constructed as a multiple way valve.

11. (amended) The apparatus of claim 1, wherein each injection valve (9.1 to 9.8) has switching possibilities to an injection port (6), to a sample loop (7), to the pumps (3, 4), to a waste collector (8) and to a separating column (11.1 to 11.8).

12. (amended) The apparatus of claim 1, wherein the separating lines (17.1 to 17.10) have a separating column and a solid phase extraction unit (13), which are coupled with further pumps (21, 22).

13. The apparatus of claim 12, wherein a multiple way valve, which can be connected with the solid phase extraction unit (23), the multi-parallel fraction output unit (24) and the waste collector (14), is disposed in the end region of the solid phase extraction unit (23).

14. (amended) The apparatus of claim 12, wherein the solid phase extraction units (23) have at least two fractionating columns each.

15. (amended) The apparatus of claim 12, wherein the solid phase extraction units (23) have between 10 and 50 fractionating columns.

16. A method for the liquid chromatograp[hic separation of substances under pressure, for which several samples, which are to be separated, are supplied simultaneously to at least several separating columns (11) and, subsequently, a detection and selection takes place simultaneously and in parallel, wherein the separating lines (17) are calibrated with respect to the retention times by means of a calibration sample and, after the individual retention times have been determined, are adjusted to the same retention time by control with flow regulators (10) on the basis of data from flow meters (12) and initial pressure meters (19).

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Atty's Docket No. 101215-63

APPLICANT : Lutz Müller-Kuhrt et al.

FILED : Concurrently Herewith

FOR : Device and Method for the Parallel Separation of Substances by Liquid Chromatography

PRELIMINARY AMENDMENT

Hon. Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Prior to examination, please amend the application as follows:

IN THE CLAIMS

Please make claims 3 - 12 solely dependent on claim 1; and

Please made claims 14 and 15 solely dependent on claim 12.

REMARKS

The above amendments were made to eliminate multiple dependent claims.

Respectfully Submitted,

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APPARATUS AND METHOD FOR THE PARALLEL, LIQUID CHROMATOGRAPHIC SEPARATION OF SUBSTANCES

The invention relates to an apparatus and to a method for the liquid chromatographic separation of substances under pressure, in accordance with the introductory portions of claims 1 and 19.

So-called chromatographic separation installations are used for the preparative and analytical separation of substance mixtures. Essentially, these installations consist in each case of a conveying unit (pump), an injection system, the actual separating device (column) and a detector. The separation of mixtures of organic components is dominated at the present time by high-pressure liquid chromatography. The reasons lie essentially in the wide range of applications and in the universality, as well as in the robustness and user friendliness of the method. It is possible to separate and detect practically any mixture of organic substances by means of high-pressure liquid chromatography. Aside from the analysis of individual samples, for which it must be possible to vary the separation parameters optimally and appropriately, there is an increasing tendency in many areas to analyze and purify large series of samples under exactly the same conditions. An exact comparability of the chromatograms and an unambiguous identification of separated substances by means of the retention times in the chromatogram are frequently needed, especially for the analytical requirements. However, unavoidable differences in the way in which chromatographic columns are filled with stationary phase material, such as the height to which the columns are filled or the packing density, can however lead to different retention times, so that an exact comparability of the chromatograms is no longer given.

Until now, for analytical and preparative purposes, individual chromatographic separation installations are used for separating individual substance mixtures. The search for pharmaceutically usable natural products and the synthesis of whole libraries of substances by means of combinatory chemistry, however, has led to more stringent requirements for the sample throughput in liquid chromatographic installations in recent times.

For example, as is well known, it is possible to process sample series consecutively by serial analyses or purification of samples. However, this procedure is very time consuming and leads to long periods of time between the processing of the first and last samples. It is a disadvantage that, in carrying out liquid chromatographic separations over longer periods of time, the constancy of the conditions cannot be guaranteed, since samples, column materials and solvents, for example, may change.

Therefore, in order to analyze a large number of samples by the so-called high throughput screening, it is desirable to be able to carry out a larger number of separations simultaneously. Present parallelized separation installations require a pumping device per separating equipment (column). As a rule, however, this is uneconomic. Moreover, the individual conveying lines of such multi-channel installations exhibit retention times, which deviate from one another.

High pressure chromatographic installations are known, for which, with a total of seven pumps, one column carousel with sixteen columns, four individual detectors and one fraction collector, a maximum of four samples can be processed in parallel (Laborpraxis, December 1967, pp 61-63). In addition, because of their expensive construction in comparison to the small number of samples, which can be processed, it is not possible to work economically.

A further installation is known, with which the maximum number of samples, which can be processed in parallel, also is four (Laboratory Automation News, Vol. 2, No. 2, May 1997). Four pumps operate four columns here. Substances are detected in a UV detector, which has one deuterium lamp and four flow cells, at only two wavelengths, which can be set before the analysis. The peak recognition in the detector switches four fraction collectors. In principle, essentially several high-pressure liquid chromatography setups are used in parallel here. This is disadvantageously uneconomic.

A significant increase in the number of pumping lines can be achieved, if several channels are supplied in a parallel operation by a single pump or pump system, pumping at a constant rate, and a flow distribution, specified by the user, results.

However, because of the different flow relationships in the individual columns, a simple, uncontrolled parallel connection of several separating columns, which are supplied by a single pump, leads to a flow distribution, which can be predicted only with difficulty because of the different flow conditions in the individual columns. Before it is started up, each column must be measured for its flow properties and a characteristic flow resistance value must be obtained.

Similarly to a parallel resistance network in electric technology, one would be able to expect here also, with such a characteristic value, a corresponding distribution of the volume flow. This method of adjusting the flow in parallel operation cannot be used in practice, since it does not take into consideration any changes with time, such as aging and blocking processes in the column material.

In the DE 115 45 423 A1, an apparatus is described, with which up to 72 parallel separations are said to be possible. The apparatus is based on two circular and disk-shaped separating phases, which are connected with one another. The flow

of the mobile phase is reversed in the case of this apparatus. For parallel measurements, the disks are to be provided with impermeable partitions. The detection is to be accomplished in a multi-channel detector, the details of which are not described. The separation phase is supplied by two pumps and a valve tree with mobile phase and samples. This apparatus has two critical points:

- There is no detailed description of how the flows in the different channels are to be controlled when the separating columns are operated in parallel. For example, if one channel becomes blocked in the apparatus shown, the flow in the other channels, in the absence of a control system, would increase.

- Likewise, it is doubtful whether the partitions on the disks prove to be tight at higher pressures. Mixing of different samples can therefore not be excluded here.

It is an object of the invention to offer an apparatus and a method for the liquid chromatographic separation under pressure, with which a parallel separation and detection, as well as a purification, if required, of at least several samples is possible, the apparatus having a compact, cost-saving construction.

This objective is accomplished with the characterizing portions of claims 1 and 6.

Advantageous further developments are given in the dependent claims.

The invention has several advantages. Significantly more samples can be separated, analyzed and purified in parallel per unit time. In the same time, in which a conventional high pressure, liquid chromatographic installation can separate only one sample or one of the above-described parallel chromatographic devices can separate four samples, the inventive equipment can separate, analyze and purify five or significantly more samples. Advantageously, in the case of the inventive

apparatus, each separating line, including the separating columns, is separated physically from the others, so that mixing of the samples cannot take place. For an operation with a low pressure gradient, only one pump is required even for a parallel operation with significantly more than five separating columns or for the high pressure gradient, a maximum of two pumps are required and, for the operation of the solid phase extraction unit, also only two pumps are required. This saves space and reduces costs. Since multi-way valves are connected in parallel for the sample injection, only one valve-control system is required. Such a parallel chromatographic apparatus, operated in parallel, can advantageously be equipped with a single multi-channel detector, instead of many individual detectors. Finally, the chromatograms of the individual separating lines are absolutely comparable with one another by the installation of a flow control system, which can be calibrated.

The invention is described in greater detail by means of examples and of drawings, in which

Figure 1A shows a flow diagram with eight separating lines, as well as one variation of the flow control unit,

Figure 1B shows a flow diagram with a further variation of the flow control unit,

Figure 2 shows a diagram of the mode of operation of the flow control and

Figure 3 shows a diagrammatic, perspective representation of the apparatus with 96 separating lines,

Figure 4 shows a diagrammatic representation of the apparatus with ten solid phase extraction units, each of which has six fractionating columns and

Figure 5 shows a diagrammatic representation of the apparatus with two fractionating columns for each solid phase extraction unit.

The samples, which are to be separated, are in sample vessels. Pursuant to a preferred embodiment of the invention, these are, for example, microtiter plates 15 in Figure 3. By means of a multi-parallel sample holding system 5, which may be constructed, for example, as an autosampler, eight samples are taken up simultaneously and supplied to the injection system 18, which consists of injection ports 6, injection valves 9 and sample loops 7 (Figures 1A, 1B). Through appropriate adjustment of the injection valve 9, excess sample material reaches the sample waste collector 8. If all eight sample delivery loops 7.1 to 7.8 are filled, all injection valves 9.1 to 9.8 are switched simultaneously and, in this manner, the sample loops 7.1 to 7.8, which are filled with samples, are connected with the separating columns 11.1 to 11.8, so that the samples are added in parallel and simultaneously to the separating columns 11.1 to 11.8. The separating columns 11.1 to 11.8 are disposed compactly in a separating column battery 11.

Over valves 1.1 to 1.4 and 2.1 to 2.4 and the pumps 3 and 4, the mobile phase is pumped over a pressure sensor 19, which is part of the flow control unit, into the individual separating lines 17.1 to 17.8. A low pressure gradient, as well as a high pressure gradient can be employed. In the case of the low pressure variant, the gradient is produced in a mixing chamber and pumped with a single pump. In the case of the high pressure gradient operation (Figure 3), the mobile phase is brought together by means of two pumps 3 and 4 on the high pressure side. The mobile phase, pumped by pumps 3 and/or 4, flows over the distribution 20 to the flow regulator 10 and transports the sample, in accordance with Figure 1A, from the sample delivery loops 7 to the respective separating column 11. The components of the samples are separated in the known manner on separating columns 11.1 to 11.8.

After the separation, the components are supplied to a multi-channel detector 13. The multi-channel detector 13 may be based on the principle of known detection methods, such as ultraviolet absorption, fluorescence spectroscopy, light scattering detection or mass detection. The multi-channel detector 13 records a separate chromatogram or spectrum for each of the eight samples.

If the inventive equipment is used exclusively for analytical determinations, the sample residues and the mobile phase are subsequently transferred to a waste collector 14.

In the case of a preparative or semi-preparative operating mode, the samples are collected after the separation and processed further. Instead of the waste collector 14, a multi-parallel fraction collector 24 is then installed. In this case, a non-destructive detector, such as a multi-parallel ultraviolet absorption detector 13 with peak recognition, controls the fraction collector, which collects the purified components. A solid phase extraction unit 23 (see Figures 4 and 5) may be installed in front of the fraction collector 24 for purifying the fractions and transferring the fractions into an organic solvent.

Especially in the case of an analytical objective, exact comparability of the chromatograms for the unambiguous identification of separated substance by means of the retention times in the chromatogram is frequently necessary. Flow control is indispensable for this application.

The flow control unit consists of the total pressure sensor 19, the flow controller 10 and the flow meter 12. In Figure 1A, flow controllers 10 are provided in front of the injection valve 9 in each parallel separating line 17.1 to 17.8. Flow meters 12 are disposed here, for example, after the detector 13. The necessary total pressure meter 19 is located between the pumps 3, 4 and the distribution 20 to the individual separating lines.

In Figure 1B, a different arrangement is provided, by way of example, in which the parts of flow regulator 10 and flow meter 12 of the flow control unit are inserted compactly before the injection valve 9.

An identical flow in all separating columns 11.1 to 11.8 does not, however, guarantee the similarity of chromatograms of the same samples. Slight differences in the way in which the separating columns 11.1 to 11.8 are filled with the stationary phase material, which are attributable, for example, to columns filled to a different height or packed to a different density, can lead to different retention times for one and the same substance. Since the flows in the individual parallel separating lines 17.1 to 17.8 can be controlled individually, they can be adjusted advantageously and pursuant to the invention, so that the slight differences in the separating columns 11.1 to 11.8 are equalized. The adjustment is made by adding a calibrating component to all separating columns 11.1 to 11.8. The different retention times are measured by one detector. After the retention times are measured, the flow for the individual separating lines 17.1 to 17.8 is calculated and adjusted, so that the same retention times result in all the separating lines 17.1 to 17.8 for the calibration component.

The two methods for adjusting a flow, required for equalizing retention times and calculated in advance, are described in greater detail in the following.

Method 1 (with pressure-controlled pumping unit):

The flow meters 12.1 to 12.8 determine the actual volume flow for each separating line 17. The flow controller 10 compares this actual value with a nominal value, specified by the evaluating and control unit 16, and, with the calculated control difference, directly controls the required volume flow for the respective separating lines 17.1 to 17.8. Aside from monitoring the specification of the nominal value, the evaluating unit 16 also monitors the controller parameters.

This procedure for adjusting the volume flows for the parallel operation of separating columns is possible when the mobile phase is supplied with pressure-controlled HPLC pumps. This supplying with mobile phase is used infrequently. The difficulty in selecting a suitable pre-pressure, which depends on the subsequent column battery, makes itself felt here.

In high pressure liquid chromatography, pumps, pumping at a constant volume, are generally used.

Method 2 (with a pumping unit controlled by the volume flow):

If the mobile phase is supplied at a constant volume flow, the latter is adjusted by a special method. The above-mentioned method permits parallel volume flows to be adjusted without mutually affecting the separating lines over the total pressure. In addition, the total volume flow is distributed here completely to the individual separating lines. The volume flow values in the individual separating lines 17.1 to 17.8 are detected by flow meters. A total pressure meter 19 determines the pressure at the output side of the pumps 3 and 4. The ratio of the total pressure to the actual volume flow value in the respective separating line represents an actual value for the flow regulator. The flow regulator 10 (such as a controller with valve) compares this actual value with a nominal value specified by the evaluating and control unit 16 and, with the calculated control difference, indirectly controls the volume flow for the respective separating line 17.1 to 17.8. In a preferred embodiment of the invention, the volume flow is determined indirectly over the pressure drop (differential pressure) at a measurement capillary.

In Figure 2, the adjusting process for four parallel HPLC separating lines 17.1 to 17.4 is illustrated in a diagram. After the HPLC pumps 3 and 4 are started, a different volume flow commences in each of the four separating lines 17.1 to 17.4. After the flow control is switched on and a common nominal value is preset,

an identical volume flow exists in the separating lines 17.1 to 17.4 after a short start-up phase.

To match the retention times, a suitable standard substance is injected simultaneously into all separating lines 17 and the retention time is determined with the help of the multi-channel detector 13. From this, the evaluating and control unit 16 calculates the necessary nominal values using a special algorithm and passes these on to the flow regulating unit. The retention times of the standard substance are checked at regular intervals in order to adjust the nominal values, if necessary. Advantageously, the flow control unit also makes an error recognition possible. If the adjusted value of the flow controller in a separating line 17 deviates from a permissible range, a system error (such as a blocked column or capillary, a leak) is recognized immediately and the separating line 17 in question is disconnected. The evaluating unit 16 signals a corresponding failure report.

The diagrammatic representation of the apparatus, shown in perspective in Figure 3, shows an apparatus expanded to 96 chromatographic channels. The multi-parallel sample holding system 5 can hold 96 samples simultaneously here.

For semi-preparative and preparative applications, a multi-parallel solid phase extraction unit 23 and a multi-parallel fraction collector of Figures 4 and 5 are coupled to the chromatographic channels.

According to Figure 4, ten samples are taken up and supplied to the separating columns 11.1 to 11.10 by means of a multi-parallel sample holding system 5, which may be constructed, for example, as an autosampler. A solvent mixture is pumped over a distributor to the ten separating lines 17.1 to 17.10 shown here by means of a pump system, consisting of the pumps 3 and 4. Flow controller units, consisting of the valves 10 and the flow meters 12 and a pressure meter 19 (not shown here), as well as an appropriate computer with a flow-control program, are disposed

here to ensure uniform flow in all separating lines 17.1 to 17.10. In each separating line 17.1 to 17.10, the solvent mixture is supplied over the sample holding system 5. Subsequently, the samples are passed on to the separating columns 11.1 to 11.10 to a parallel multi-channel detector 13. Water is supplied by pump 21 to all separating lines 17.1 to 17.10 in order to increase the polarity of the mixture and, with that, to make possible the extraction of the sample components on the adjoining solid phase extraction unit 23. For each separating line 17.1 to 17.10, the solid phase extraction unit 23 contains six fractionating columns here.

In the variation of Figure 5, two fractionating columns are provided in combination with a 10-port, two-position valve in each of the separating lines 17.1 to 17.10. The pump 22 is used to equilibrate the solid phase extraction unit 23 for cleaning the samples and finally for transferring the samples to the fraction collector 24.

List of Reference Symbols

1.1 to 1.4	valve
	mobile phase
	supply A
2.1 to 2.4	valve
	mobile phase
	supply B
3	pump
4	pump
5	sample holding system
6	injection port
7	sample loop
7.1 to 7.8	sample loops
8	sample waste collection
9	injection valve
9.1 to 9.8	injection valves
10	flow regulator
11	battery of separating columns
11.1 to 11.10	separating columns
12	flow meter
13	detector
14	waste collector
15	microtiter plate
16	evaluation and control unit
17.1 to 17.10	separating lines
18	injection system
19	total pressure meter
20	distributor
21	pump
22	pump
23	solid phase extraction unit 23.1 to 23.10
24	fraction collector

Claims

1. An apparatus for the liquid chromatographic separation of substances under pressure, wherein at least several liquid chromatographic separating lines (17), which are disposed in parallel, are supplied by a single pumping unit (one or two pumps) and, in the region, when the samples are supplied, are combined with a sample delivery system (5) and an injection system (18) as well as, in the detection region, with a detector (13), connected with an evaluation and control unit (16).

2. The apparatus of claim 1, wherein the liquid chromatographic separation lines (17) have flow control units (10, 12, 12.1, 19).

3. The apparatus of claims 1 or 2, wherein the flow control units (10, 12, 19) consist of flow regulators (10), a total pressure meter (19) and flow meters (12).

4. The apparatus of claims 1 to 3, wherein the flow regulating units (10, 12, 19) in each separating line (17) can be controlled by software and/or hardware.

5. The apparatus of claims 1 to 4, wherein flow regulators (10) and flow meters (12) are disposed at different places in a separating line (17).

6. The apparatus of claims 1 to 5, wherein flow regulators (10) and flow meters (12) are disposed compactly in one place in the separating line (17).

7. The apparatus of claims 1 to 6, wherein the flow regulator unit (10, 12, 19) is disposed in front of or behind the separating columns (11.1 to 11.8).

8. The apparatus of claims 1 to 5, wherein the total pressure meter (19) is disposed on the output side of the pump (3, 4).

9. The apparatus of one of the claims 1 to 8, wherein the sample holding system (5) is connected with at least several parallel sample holding lines over at least several injection ports (6) and injection valves (9) and sample loops (7) of the multi-parallel injection system (18) are connected with at least several separating columns (11.1 to 11.8), which are coupled with a detector (13), which has several determination channels.

10. The apparatus of one of the claims 1 to 9, wherein the separating columns (11.1 to 11.8) are combined compactly into a battery of separating columns (11).

11. The apparatus of one of the claims 1 to 10, wherein each injection valve (9) is disposed before the separating columns (11.1 to 11.8).

12. The apparatus of one of the claims 1 to 11, wherein each injection valve (9) is constructed as a multiple way valve.

13. The apparatus of one of the claims 1 to 12, wherein each injection valve (9.1 to 9.8) has switching possibilities to an injection port (6), to a sample loop (7), to the pumps (3, 4), to a waste collector (8) and to a separating column (11.1 to 11.8).

14. The apparatus of one of the claims 1 to 13, wherein the separating lines (17.1 to 17.10) have a separating column and a solid phase extraction unit (13), which are coupled with further pumps (21, 22).

15. The apparatus of claims 14 o4 15, wherein the liquid chromatographic separating lines (17.1 to 17.10) have flow control units (10, 12, 19).

16. The apparatus of claims 14 or 15, wherein a multiple way valve, which can be connected with the solid phase extraction unit (23), the multi-parallel fraction output unit (24) and the waste collector (14), is disposed in the end region of the solid phase extraction unit (23).

17. The apparatus of one of the claims 14 to 16, wherein the solid phase extraction units (23) have at least two fractionating columns each.

18. The apparatus of one of the claims 14 to 17, wherein the solid phase extraction units (23) have between 10 and 50 fractionating columns.

19. A method for the liquid chromatographic separation of substances under pressure, wherein several samples, which are to be separated, are supplied simultaneously to at least several separating columns (11) and subsequently detected and selected simultaneously and in parallel.

20. The method of claim 19, wherein the separating lines (17) are calibrated with respect to the retention times by means of a calibration sample and, after the individual retention times have been determined, are adjusted to the same retention time by control with flow regulators (10) on the basis of data from flow meters (12) and initial pressure meters (19).

21. The method of claim 20, wherein the ratio of the total pressure to the volume flow to the respective separating line is used as actual value for indirectly controlling the volume flow.

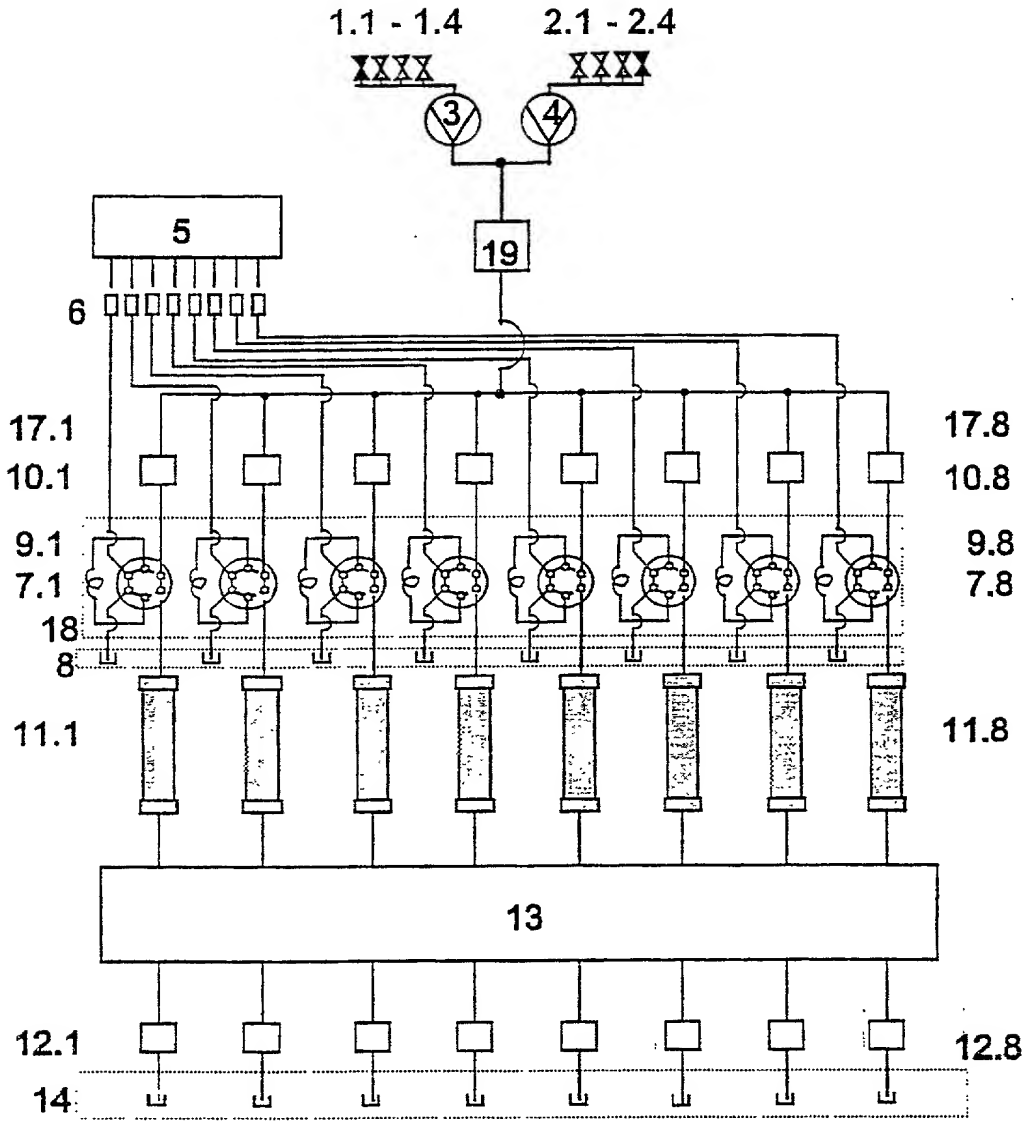
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Fig. 1A

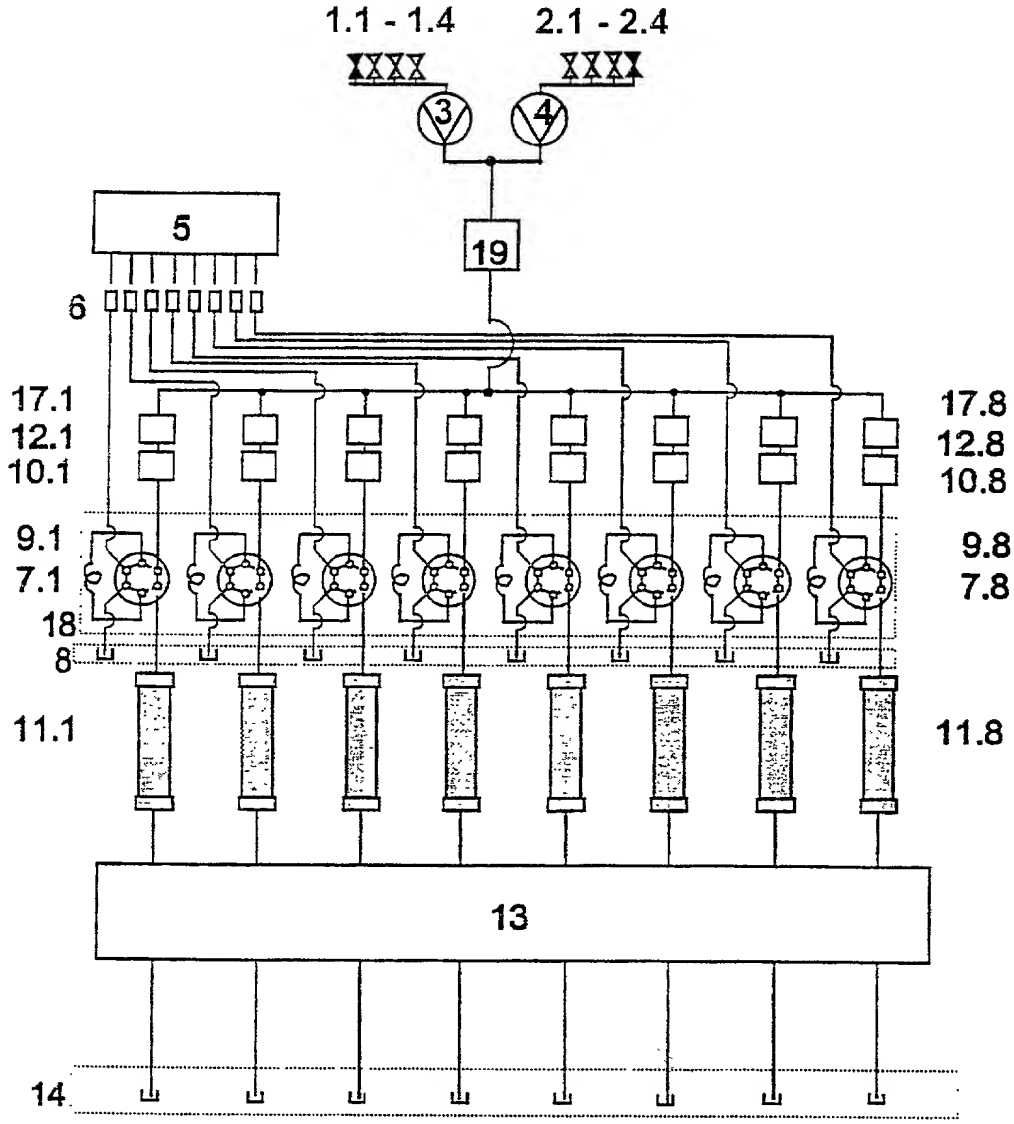
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Fig. 1B

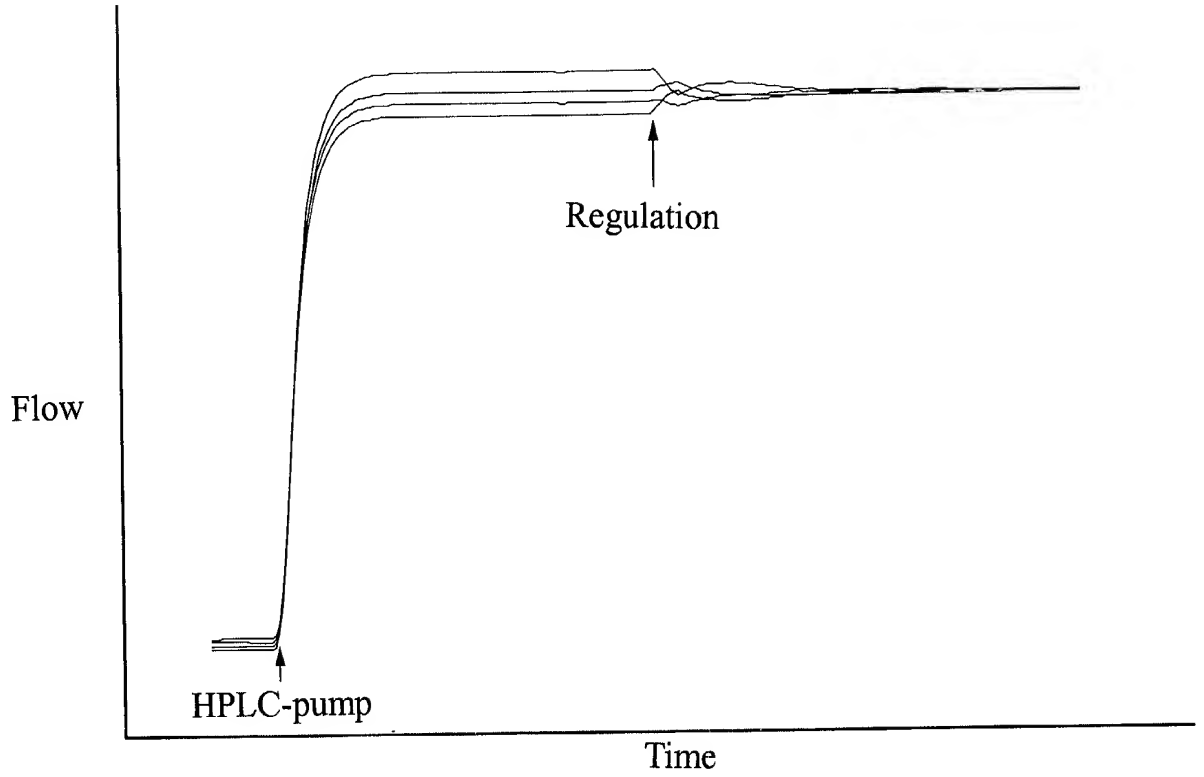

Fig. 2/3

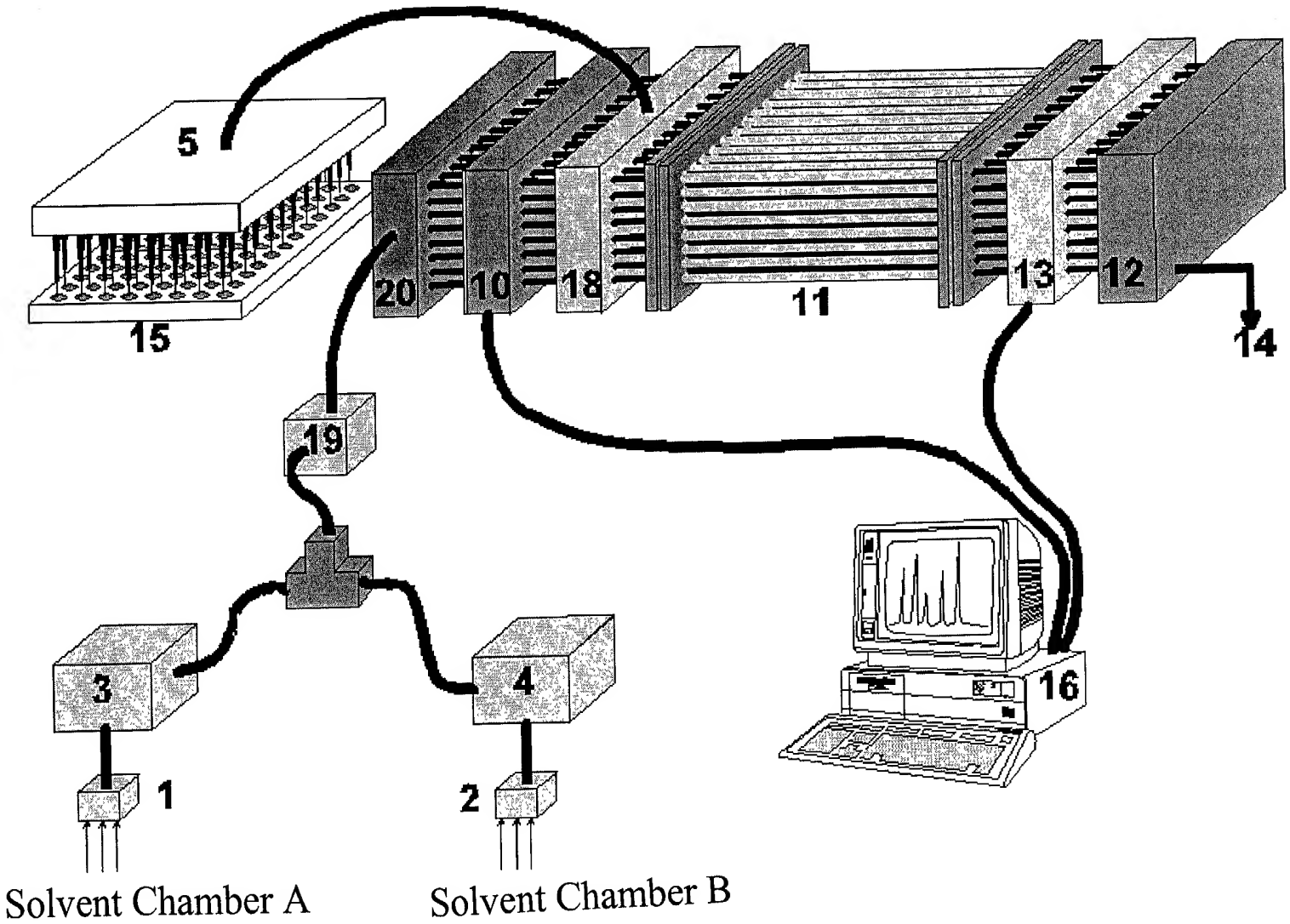

Fig. 3

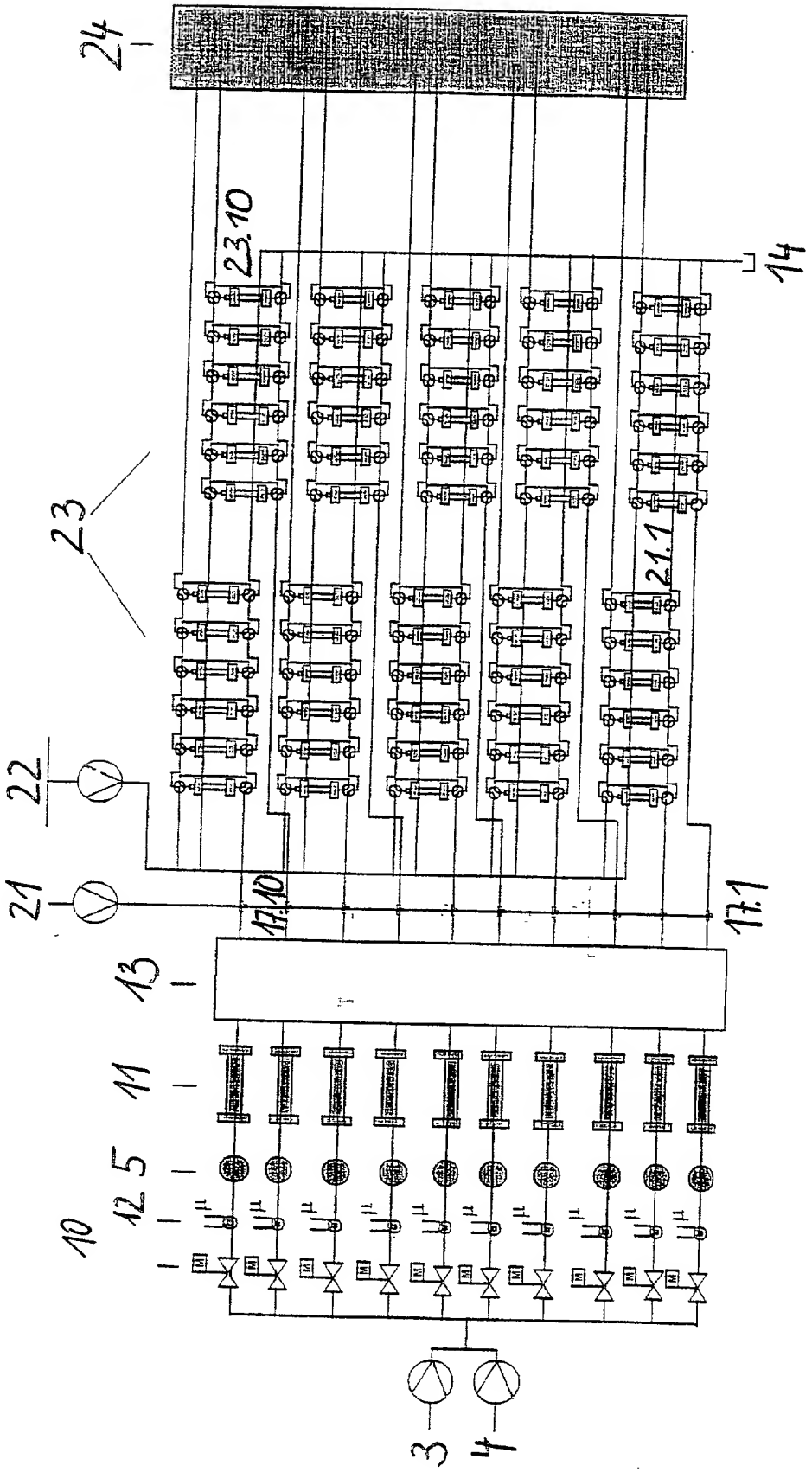

Fig. 4

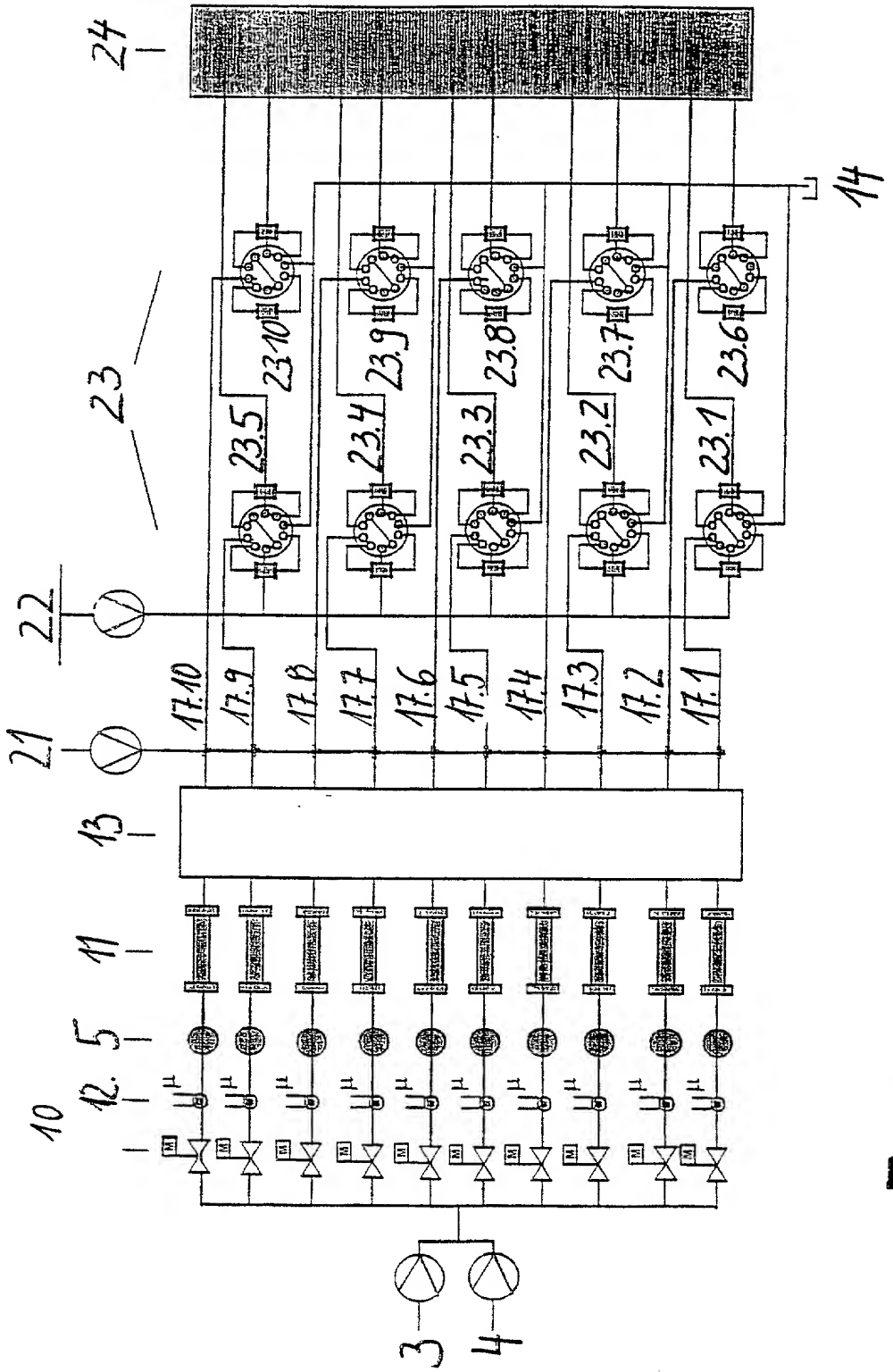
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Fig.5

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If each inventor understands English, the Declaration and Power of Attorney below is suitable for use when filing a regular patent application and also when entering the national stage, in the case of an International application designating the USA under the PCT.

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION	Attorney Docket No. 101215-63

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name,
I believe I am the original, first and sole inventor (if only one name is listed below at 201) or an original, first and joint inventor (if plural names are listed below at 201-205) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Device and Method for the Parallel Separation of Substances by Liquid Chromatography

the specification of which (check one)

____ is attached hereto

__✓__ was filed on _____22 November 1999_____

under Serial Number __PCT/EP99/09747___ and was amended on ____Jan. 23, 2001____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56.

I list below any prior foreign application(s) for patent or inventor's certificate in respect of which foreign priority benefits are claimed under 35 USC 119; and any prior foreign application(s) for patent or inventor's certificate in respect of which such foreign priority rights are not claimed and which has a filing date before that of any application in respect of which such foreign priority benefits are claimed:

Application Number	Country	Filing Date (day, month, year)	Priority Claimed under 35 USC 119
198 55 001.4	Germany	20 November 1998	YES:__✓_ NO:____
299 10 725.6	Germany	14 June 1999	YES:__✓_ NO:____
			YES:____ NO:____

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

Application No.	Filing Date

Combined Declaration and Power of Attorney
101215-63
Page 2

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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	City of Residence	State or Foreign Country	Country of Citizenship
	Post Office Address	City	State & ZIP/Country

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 201	Date 5.7.01
Signature of Inventor 202	Date 12.7.01
Signature of Inventor 203	Date 29/06/01
Signature of Inventor 204	Date 2.7.01
Signature of Inventor 205	Date